FORM PTO-1390 (REV. 11-2000) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

# TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

| ATTORNEY'S DOCKET NUMBER | |
|---|---|
| QGN-020.0P-US | |
| U S APPLICATION NO (If known, see 37 CFR 1 5) | |
| 09/889093 (not yet assigned) | |
| INTERNATIONAL APPLICATION NO. | INTERNATIONAL FILING DATE |
| PCT/EP00/00052 | 05 - January - 2000 |
| PRIORITY DATE CLAIMED | |
| 11 - January - 1999 | |

TITLE OF INVENTION

METHOD FOR ISOLATING DNA FROM BIOLOGICAL MATERIALS

APPLICANT(S) FOR DO/EO/US

Max-Planck-Gesellschaft Zur Forderung Der Wissenschaften E.V. and Qiagen GmbH

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [X] The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is attached hereto (required only if not communicated by the International Bureau).
   b. [ ] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [X] is attached hereto.
   b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [ ] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). v "unexecuted"
10. [X] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

**Items 11 to 20 below concern document(s) or information included:**

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [ ] Other items or information:

| U.S. APPLICATION NO (if known, see 37 CFR 1.5) | INTERNATIONAL APPLICATION NO | ATTORNEY'S DOCKET NUMBER |
|---|---|---|
| 09/889093 | | |

| 21. ☒ The following fees are submitted: | CALCULATIONS PTO USE ONLY | |
|---|---|---|
| **BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):** | | |
| Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... **$1000.00** | | |
| International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..✔..... **$860.00** | | |
| International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... **$710.00** | | |
| International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... **$690.00** | | |
| International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... **$100.00** | | |
| **ENTER APPROPRIATE BASIC FEE AMOUNT =** | $ 860.00 | |
| Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)). | $ | |

| CLAIMS | NUMBER FILED | NUMBER EXTRA | RATE | $ | |
|---|---|---|---|---|---|
| Total claims | 20 - 20 = | ----- | x **$18.00** | $ | ----- |
| Independent claims | 3 - 3 = | ----- | x **$80.00** | $ | ----- |
| MULTIPLE DEPENDENT CLAIM(S) (if applicable) | | | + **$270.00** | $ | ----- |
| **TOTAL OF ABOVE CALCULATIONS =** | | | | $ 860.00 | |
| ☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. + | | | | $ | |
| **SUBTOTAL =** | | | | $ 860.00 | |
| Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). | | | | $ | |
| **TOTAL NATIONAL FEE =** | | | | $ 860.00 | |
| Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property + | | | | $ | |
| **TOTAL FEES ENCLOSED =** | | | | $ 860.00 | |
| | | | | **Amount to be refunded:** | $ |
| | | | | **charged:** | $ |

a. ☒ A check in the amount of $ 860.00 to cover the above fees is enclosed. (check no. 3566)

b. ☐ Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-0268 . A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO.

Leon R. Yankwich
YANKWICH & ASSOCIATES
130 Bishop Allen Drive
Cambridge, MA 02139

Leon Zwicker
SIGNATURE

Kenneth P. Zwicker
NAME

43,310
REGISTRATION NUMBER

**PATENT COOPERATION TREATY**
**IN THE UNITED STATES DESIGNATED/ELECTED OFFICE**

| | |
|---|---|
| Application of: | Müller et al. |
| Serial No.: | (not yet assigned) |
| Filed: | (concurrently herewith) |
| Entitled: | METHOD FOR ISOLATING DNA FROM BIOLOGICAL MATERIALS |

ART UNIT: (not yet assigned)

EXAMINER: (not yet assigned)

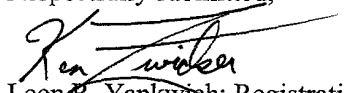
**National Stage of International Appln. No. PCT/EP00/00052, filed 05 January 2000**
Attorney Docket No.: QGN-020.0P US

Asst. Commissioner of Patents and Trademarks
**Box PCT**
Washington, D.C. 20231

**NATIONAL STAGE APPLICATION TRANSMITTAL:**

1. Designated/Elected Office (DO/EO/US) Transmittal Form PTO-1390 (2 pages).
2. Duplicate copy Transmittal Form PTO-1390 page 2 fee calculation/authorization.
3. Copy of International Application as published **22** pages
   (including 1 drawing & International Search Report).
4. English language translation of the International Application as filed **21** pages
   (including 1 drawing & International Search Report).
5. English language translation of the International Preliminary Examination Report Annex **1** page.
6. Unexecuted Oath/Declaration and Power of Attorney (3 pages).
7. Preliminary Amendment.
8. Information Disclosure Statement (IDS; including form PTO-1449).
9. Copy of references cited in IDS.
10. Return receipt post card.
11. Check No. **3566** in the amount of $ **860.00** to cover national application filing fee.

Respectfully submitted,



Leon R. Yankwich; Registration No. 30,237
Kenneth P. Zwicker, PhD; Registration No. 43,310
Attorneys for Applicant
YANKWICH & ASSOCIATES
130 Bishop Allen Drive
Cambridge, Massachusetts 02139
telephone: (617) 491-4343
telefax: (617) 491-8801

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

The undersigned hereby certifies that this correspondence listed above is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" Service under 37 CFR §1.10, postage prepaid, Express Mailing Label No. **EL 164335248 US**, in an envelope addressed to the Asst. Commissioner for Patents, Box PCT, Washington, D.C. 20231 on the date indicated below.

July 10, 2001
date

Stephanie L. Leicht
Stephanie L. Leicht

**PATENT COOPERATION TREATY**
**IN THE UNITED STATES DESIGNATED/ELECTED OFFICE**

| | | |
|---|---|---|
| Application of: | Müller et al. | |
| Serial No.: | (not yet assigned) | ART UNIT: (not yet assigned) |
| Filed: | (concurrently herewith) | EXAMINER: (not yet assigned) |
| Entitled: | METHOD FOR ISOLATING DNA FROM BIOLOGICAL MATERIALS | |

**National Stage of International Appln. No. PCT/EP00/00052, filed 05 January 2000**
Attorney Docket No.: QGN-020.0P US

Asst. Commissioner of Patents and Trademarks
**Box PCT**
Washington, D.C. 20231

Sir/Madam:

**PRELIMINARY AMENDMENT OF THE**
**NATIONAL STAGE APPLICATION FILED UNDER 35 U.S.C. 371**

This paper is filed concurrently with the national stage filing of an application under 35 U.S.C. 371 and 37 CFR 1.495 corresponding to International Application No. PCT/EP00/00052, filed 05 January 2000. Please enter the following amendments prior to calculation of the filing fee and prior to examination on the merits.

IN THE SPECIFICATION

Please amend the following specification section under the provisions of 37 CFR §1.121(b)(2)(i) & (ii) so that it appear as follows:

On page 1, after the title "Method for isolating DNA from biological materials", please insert the following section:

--CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a national stage application of PCT/EP00/00052, filed 05 January 2000, which claims priority to German application DE 199 00 638.5, filed 11 January 1999, the entirety of which is hereby incorporated by reference.--

IN THE CLAIMS

Please cancel original claims 1-14 and add new claims 15-34 under the provisions of 37 CFR §1.121(c)(1)(i) so that they appears as follows:

--15. (new) A method for isolating a nucleic acid from a biological sample comprising the steps of:

(a) providing an extraction buffer comprising a phenol-neutralizing substance, wherein said extraction buffer

(i) has a pH from about 2 to about 8, and

(ii) has a salt concentration of at least about 100 mM;

(b) contacting said extraction buffer with a biological sample containing nucleic acid, and contacting said biological sample with an adsorption matrix; and

(c) isolating said nucleic acid from said adsorption matrix. --

--16. (new) The method of claim 15, wherein said extraction buffer has a pH from about 4 to about 6.5. --

--17. (new) The method of claim 15, wherein said extraction buffer comprises at least one salt from the group consisting of KCl and NaCl. --

--18. (new) The method of claim 15, wherein said phenol-neutralizing substance comprises at least about 0.5% polyvinylpyrrolidone. --

--19. (new) The method of claim 15, wherein said adsorption matrix comprises an insoluble carbohydrate. --

--20. (new) The method of claim 19, wherein said adsorption matrix comprises a component of potato flour. --

--21. (new) The method of claim 15, wherein said biological sample comprises fecal material. --

--22. (new) The method of claim 15, wherein said extraction buffer is incubated with said biological sample before contacting said extraction buffer and said biological sample with said adsorption matrix. --

--23. (new) The method of claim 22, wherein said incubation occurs at a temperature of less than or equal to about 10°C. --

--24. (new) The method of claim 22, wherein said incubation comprises at least one treatment regime selected from the group consisting of chemical treatment, thermal treatment, and enzymatic treatment. --

--25. (new) The method of claim 22, wherein said incubation occurs at a temperature of greater than or equal to about 50°C. --

--26. (new) The method of claim 15, wherein contacting said biological sample with said adsorption matrix occurs under at least one physical condition selected from the group consisting of centrifugation, reduced pressure, and gravity. --

--27. (new) The method of claim 24, wherein contacting said biological sample with said adsorption matrix occurs under at least one physical condition selected from the group consisting of centrifugation, reduced pressure, and gravity. --

--28. (new) An extraction buffer useful to isolate a nucleic acid from a biological sample comprising a phenol-neutralizing substance, wherein said extraction buffer

(i) has a pH from about 2 to about 8, and

(ii) has a salt concentration of at least about 100 mM. --

--29. (new) The extraction buffer of claim 28, wherein said extraction buffer has a pH from about 4 to about 6.5. --

--30. (new) The extraction buffer of claim 28, wherein said extraction buffer comprises at least one salt from the group consisting of KCl and NaCl. --

--31. (new) The extraction buffer of claim 28, wherein said phenol-neutralizing substance comprises at least about 0.5% polyvinylpyrrolidone. --

--32. (new) A kit for isolating a nucleic acid from a biological sample comprising:

(a) an extraction buffer according to any one of claims 28-31, and

(b) an adsorption matrix. --

--33. (new) The kit of claim 32, wherein said adsorption matrix comprises an insoluble carbohydrate. --

--34. (new) The kit of claim 33, wherein said adsorption matrix comprises a component of potato flour. --

## REMARKS

This paper is being filed concurrently with Applicants' transmittal of the application and related papers necessary to request entry into the national stage under 35 U.S.C. 371 and 37 CFR 1.495 on the basis of the International Application No. PCT/EP00/00052, filed 05 January 2000.

The specification has been amended to provide updated information regarding cross-references to related applications under 37 CFR §1.78.

Applicants respectfully request that pending claims 1-14 of the international application be canceled and new claims 15-34 be entered as noted above.

New claims 15-27 and 32-34 correspond to original claims 1-14 in scope and subject matter but are written in proper claim format for review in the United States Patent and Trademark Office. It is not the intention of the Applicants to abandon or otherwise surrender any of the inventive subject matter disclosed in the application as originally filed.

New claims 28-31 are directed to a novel extraction buffer useful to isolate nucleic acid from a biological sample, as taught in the specification as filed as an object of the present invention. Support for these added claims can be found throughout the specification as filed. See, specifically, page 3, line 15 bridging to page 4, line 18. No new matter is introduced.

An information disclosure statement pursuant to 37 CFR §1.97 and §1.98 is submitted concurrently herewith in order to expedite consideration of this application on the merits. Pursuant to 37 CFR §1.98(a)(2), copies of each of the citations are submitted with the accompanying papers.

A check for the filing fees accompanies these papers, and the fees are calculated taking the foregoing amendments into account. The Commissioner is specifically authorized to charge any additional fees deemed to be necessary in connection with the filing of this paper or any of the accompanying papers, or any other fees necessary to complete national stage filing based on said

international application, to Deposit Account 50-0268. This authorization to charge Deposit Account 50-0268, extends, in particular, to any national stage fee set forth under 37 CFR 1.492.

Examination and allowance of the claims as presented herein are respectfully solicited.

Respectfully submitted,

Leon R. Yankwich; Registration No. 30,237
Kenneth P. Zwicker, PhD; Registration No. 43,310
Attorneys for Applicants
YANKWICH & ASSOCIATES
130 Bishop Allen Drive
Cambridge, Massachusetts 02139
telephone: (617) 491-4343
telefax: (617) 491-8801

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

The undersigned hereby certifies that this correspondence listed above is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" Service under 37 CFR §1.10, postage prepaid, Express Mailing Label No. **EL 164335248 US**, in an envelope addressed to the Asst. Commissioner for Patents, Box PCT, Washington, D.C. 20231 on the date indicated below.

July 10, 2001
Date

Stephanie L. Leicht

# Method for isolating DNA from biological materials

## Description

The invention relates to a method for the stabilization, purification or/and isolation of nucleic acids from biological materials, in particular stool samples which may contain contaminations and inhibitors or interfering substances. Furthermore, a reagent kit suitable for carrying out the method of the invention is described.

Numerous examples from various research areas verify the importance of analyzing nucleic acids from biological materials contaminated with substances which damage nucleic acids during storage and hinder an enzymatic manipulation of the nucleic acids, for example by amplification. It is therefore important for the usability of the nucleic acids contained in the biological materials for further analyses that said substances are present only at very low concentrations or are completely removed from the sample.

The analysis of nucleic acids from fecal samples is of particular importance. An important medical application is the detection of tumor-specific modifications of nuclear human DNA from stools, which may serve as parameters in the early diagnosis of tumors of the digestive tract. Likewise, the detection of bacterial and viral infectious pathogens from stool samples by nucleic acid-based assay methods becomes increasingly important.

The application of a combination of various purification steps such as protease treatment, phenol/chloroform extraction, binding of nucleic acids to silica in the presence of chaotropic salts, gel filtration, anion exchange chromatography and the use of cationic detergents is well known for the

purification of nucleic acids from stool samples. However, the nucleic acids isolated from stool samples using said methods are generally unstable and often cause problems in subsequent enzymatic reactions such as, for example, PCR. The reason for this are substances which are isolated together with the nucleic acid and which damage said nucleic acid and also inhibit enzymatic reactions. Inhibitor classes contained in stools, where known, are hemoglobin and its metabolites, bile acids and bile acid derivatives and also polysaccharides.

PCT/EP/96/03595 describes a method for purifying, stabilizing or/and isolating nucleic acids from biological materials, in particular feces, in which an adsorption matrix for binding contaminations is added to a nucleic acid-containing sample from biological materials. The adsorption matrix used is preferably carbohydrate-based, for example starch, cellulose, glycogen or/and other biogenic or nonbiogenic carbohydrates or mixtures thereof, with flours made of cereals, peas, corn, potatoes or components thereof or mixtures being preferred. Mixtures of purified carbohydrates or/and flours, in particular mixtures of cellulose and potato flour, have proved particularly suitable for purifying nucleic acids from stool samples.

In some cases however, the nucleic acid-damaging substances and PCR inhibitors are not completely removed when using the method described in PCT/EP96/03595. In the case of a - variable - proportion of inhibitory stool samples, the enzymatic treatment of the nucleic acids following purification using the standard protocol is not possible.

It was therefore an object of the present invention to provide a method for purifying nucleic acids, which removes at least some of the disadvantages of the prior

art and which in particular makes it possible to reproducibly purify nucleic acids from "inhibitory samples".

Surprisingly, it was found that purification of nucleic acids can be improved even from inhibitory samples when taking one or more of the measures mentioned below:

(a) using an extraction buffer having an acidic to neutral pH,

(b) using an extraction buffer having a high salt content and

(c) using an extraction buffer containing a phenol-neutralizing substance.

The invention therefore relates to a method for the purification, stabilization or/and isolation of nucleic acids from biological materials, in which an extraction buffer and an adsorption matrix for binding contaminations are added to the nucleic acid-containing sample and the nucleic acids are subsequently removed from the adsorption matrix, and contaminations bound thereto, the extraction buffer containing

(a) a pH in the range from 2-8,

(b) a salt concentration of at least 100 mM or/and

(c) a phenol-neutralizing substance.

In a first embodiment, the buffer has a pH in the range from 2 to 8, preferably from 3 to 7 and particularly preferably from 4 to 6.5. The use of acetate buffers, for example Na acetate, has proved beneficial here. However, it is also possible to use other buffers, for example phosphate buffers or citrate buffers.

According to a second embodiment, the extraction buffer contains a salt concentration of at least 100 mM, preferably of at least 200 mM up to the maximum solubility of the salt used in each case. The preferred salt used is an alkali metal halide, for example NaCl or KCl or mixtures thereof.

According to a third embodiment, the buffer contains at least one phenol-neutralizing substance. Preferred examples of substances which can neutralize phenols are polyvinylpyrrolidone (PVP) of various polymerization grades, e.g. PVP-10, reducing agents, e.g. thiol reagents such as β-mercaptoethanol or dithiothreitol or borates. Particular preference is given to using polyvinylpyrrolidone at a concentration of at least 0.5% (w/w) up to the solubility limit.

Furthermore, the extraction buffers suitable for the method of the invention preferably contain a chelator such as EDTA, for example, or/and a detergent, for example an ionic detergent such as SDS. The chelator is present preferably at a concentration of 1 to 200 mM. The detergent concentration is preferably from 0.1 to 5% (w/w).

The adsorption matrix is such that it can, in combination with the extraction buffer, remove or neutralize contaminations which lead to damage of nucleic acids or/and prevent enzymatic reactions from being carried out or/and inhibit enzymatic reactions, examples of which are degradation products of hemoglobin, for example bilirubin and its degradation products, bile acids or salts thereof or their degradation products or/and polysaccharides and polyphenols, in particular of plant origin. Preference is given to using an insoluble adsorption matrix.

With respect to the suitable adsorption matrices, reference is made to the application PCT/EP96/03595. Particular preference is given to using carbohydrate-based adsorption matrices, for example flours made of cereals, corn, peas, soybean and in particular of potatoes or components thereof or mixtures thereof. Particular preference is given to mixtures of flours

with other carbohydrates, for example purified carbohydrates such as cellulose.

The amount in which the adsorption matrix is added to the sample essentially depends on the sample composition. The adsorption matrix may be employed, for example, in a proportion by weight of from 0.05:1 to 100:1, in particular from 0.1:1 to 10:1, based on the sample.

The nucleic acid-containing sample is taken from biological materials which contain nucleic acid-degrading or enzymatic reaction-inhibiting contamination. The preferred source of the sample is feces. However, said sample may also be taken from other sources, e.g. tissues of all kinds, bone marrow, human and animal body fluids such as blood, serum, plasma, urine, sperm, CSF, sputum and swabs, plants, parts and extracts of plants, e.g. saps, fungi, microorganisms such as bacteria, fossilized or mummified samples, soil samples, sludge, waste waters and food.

Preferably, the sample is taken up in extraction buffer prior to adding the adsorption matrix and is preincubated for a period desired in each case. On the other hand, it is also possible to add sample, extraction buffer and adsorption matrix together at the same time. The extraction buffer is preferably used in a proportion by weight of at least 0.1:1, in particular of from 0.5:1 to 50:1, based on the sample. The sample may be incubated in the extraction buffer at room temperature and the incubation preferably includes a homogenization step, for example by vortexing.

In an embodiment of the invention, the incubation may be carried out under conditions which are beneficial for a release of the nucleic acids from the sample material. Such incubation conditions are used in

particular if nucleic acids from materials "difficult" to break down, for example cells such as bacteria or parasites or viruses for example, are to be detected. In this case, the release of the nucleic acids during the incubation can be improved by chemical, thermal or/and enzymatic treatment, as a result of which a higher yield of nucleic acids is obtained from the sample material, both regarding total DNA and, specifically, regarding the DNA to be detected. It is preferred here to raise the temperature, for example to ≥ 50°C, in particular to ≥ 70°C.

If, on the other hand, nucleic acids from materials easy to break down, sensitive cells such as human cells for example, are to be determined, the incubation may also be carried out at a reduced temperature, for example ≤ 10°C, in particular ≤ 4°C, in order to avoid or restrict in this way the undesired release of other nucleic acids in the sample.

After addition of the adsorption matrix, the sample is further incubated. This incubation, too, may be carried out at room temperature, at a reduced temperature or at conditions beneficial to the release of nucleic acids, depending on the requirement.

After the incubation, the adsorption matrix can be removed from the sample by centrifugation, for example. Alternatively, the adsorption matrix may be added directly to the sample, for example in the case of liquid biological samples. Furthermore, it is possible to direct the sample over an adsorption matrix by centrifugation, application of reduced pressure or/and by means of gravity, with the adsorption matrix then being preferably present in a column.

The treatment with extraction buffer and adsorption matrix leads to a significant increase in stability of the nucleic acids contained in the sample and to a

better reproducibility of the subsequent isolation of the nucleic acids. This is true in particular if the isolation is followed by enzymatic manipulation of the nucleic acids, for example an amplification or/and a restriction cleavage. Particular preference is given to carrying out the amplification, for example by PCR (polymerase chain reaction), LCR (ligase chain reaction), NASBA (nucleic acid base-specific amplification) or 3SR (self-sustained sequence replication).

As already mentioned in PCT/EP96/03595, a particularly preferred aspect of the present invention is the analysis, detection or isolation of nucleic acids, in particular DNA, from stool samples. The method of the invention makes it possible to obtain clean and amplifiable nucleic acids from fecal samples, which can be used for detecting mutations, in particular tumor-specific DNA mutations.

The present invention further relates to a reagent kit for stabilizing and purifying nucleic acids from biological materials, comprising:

(a) an extraction buffer as described above which is suitable for taking up a nucleic acid-containing sample, and

(b) an adsorption matrix for binding contaminations of the biological materials.

The adsorption matrix may be present packaged in portions, for example packed in a column such as, for example, a minicolumn which can be centrifuged. The buffer may be present in a ready-to-use form, as concentrate or as lyophilizate.

The reagent kit preferably contains additional means for purifying nucleic acids, which include, for example, mineral or/and organic support materials and, where appropriate, solutions, auxiliary substances

or/and accessories. Mineral components of support materials may be, for example porous or nonporous metal oxides or metal mixed oxides, for example aluminum oxide, titanium dioxide or zirconium dioxide, silica gels, glass-based materials, for example modified or unmodified glass particles or glass powder, quartz, zeolites or mixtures of one or more of the abovementioned substances. On the other hand, the support may also contain organic components which are selected from, for example, latex particles optionally modified with functional groups, synthetic polymers such as, for example, polyethylene, polypropylene, polyvinylidene fluoride, in particular ultra high molecular weight polyethylene or HD polyethylene, or mixtures of one or more of the abovementioned substances.

The support may be present, for example, in the form of particles having an average size of from 0.1 µm to 100 µm. When using a porous support, an average pore size of from 2 µm to 100 µm is preferred. The support may be present, for example, in the form of loose beds of particles, filtering layers, for example made of glass, quartz or ceramic, membranes, for example membranes in which a silica gel has been arranged, fibers or tissues of mineral support materials, such as, for example quartz or glass wool and also in the form of latices or frit materials of synthetic polymers.

In addition, the reagent kit of the invention may also contain auxiliary substances such as enzymes and other means for manipulation of nucleic acids, for example at least one amplification primer and enzymes suitable for amplification of nucleic acids, for example a nucleic acid polymerase or/and at least one restriction endonuclease.

The primers for amplification of nucleic acids are expediently derived from the genes to be analyzed, i.e. for example from oncogenes, tumor suppressor genes or/and microsatellite sections. Enzymes suitable for amplification of nucleic acids and restriction endonucleases are well known and commercially available.

In addition, the following figures and examples are intended to illustrate the present invention. In the figures:

Fig. 1: shows the amplificability of DNA in inhibitory stool samples using an extraction buffer of the prior art (Fig. 1a) and an extraction buffer of the invention (Fig. 1b).

**Example 1**
**Analysis of DNA from stool samples**

DNA was purified from stool samples using an adsorption matrix made of cellulose and potato flour and then amplified by means of PCR.

Human stool samples were collected, frozen and stored at -80°C. 200 mg of stools were introduced into a 2 ml microcentrifuge vessel and stored on ice. The stool sample was then taken up in 600 µl of extraction buffer and the mixture was homogenized by vortexing for 1 min.

The potato flour and cellulose-based adsorption matrix (200 mg) was taken up in 300 µl of extraction buffer and resuspended by vortexing. The matrix suspension was then added to the stool homogenate and subjected to vortexing for 1 min.

The sample was centrifuged for 5 min in order to precipitate stool particles, the adsorption matrix and other contaminations. The supernatant was transferred

to a new microcentrifuge vessel and centrifuged for a further 5 min.

The DNA contained in 600 µl of the supernatant was further purified with the aid of reagents and centrifugation columns, as described below. After proteinase K treatment, the nucleic acids were bound to a silica gel membrane of a centrifugation column in the presence of chaotropic salts and eluted after repeated washing steps.

A template (a DNA coding for GFP (green fluorescence protein)) and the other components (primers, polymerase, nucleotides, buffers) necessary for its amplification were added to the DNA eluates. The final concentration of the DNA eluates in the PCR mixture was 10% (v/v).

DNA isolates from inhibitory stool samples of a total of 19 individuals were tested for amplificability by means of PCR (lanes 1 to 19 in Fig. 1a and b). After PCR, the mixtures were fractionated by gel electrophoresis and the amplification products (expected length 771 bp) were visualized by ethidium bromide staining.

A DNA length marker (M; 1 kB Marker, Gibco BRL, Bethesda Maryland) was applied to the gel as a reference. Controls added to the GFP-PCR mixture instead of the DNA eluates were Tris buffer (T), a highly inhibitory stool DNA (I) or a non-inhibitory stool DNA (N). Moreover, in a control reaction GFP was amplified without any additions (-).

In the case of inhibitory stool samples, it was often impossible to obtain an amplification product when using the stool-dissolving buffer (500 mM Tris-HCl pH 9.0, 50 mM EDTA, 10 mM NaCl) used in PCT/EP96/03595. Thus, Fig. 1a shows that using the protocol known from

PCT/EP96/03595 an amplification took place only in two of 19 samples tested (samples No. 4 and 15).

Surprisingly, it was found that it was possible to dramatically improve the amplificability of the DNA by replacing the standard buffer with one of buffers E1 to E8 shown in Table 1 below.

Table 1

| | Na acetate | NaCl | KCl | EDTA | SDS | PVP-10 | pH |
|---|---|---|---|---|---|---|---|
| E1 | 0.2M | 2.5M | - | 60 mM | 1.5% | 2% | 6.5 |
| E2 | 0.2M | 0.5M | - | 50 mM | 1.4% | 3% | 5.0 |
| E3 | 0.1M | 1.0M | - | 60 mM | 1.0% | 4% | 6.0 |
| E4 | 0.1M | 0.5M | - | 50 mM | 1.4% | 2% | 5.5 |
| E5 | 0.3M | - | 0.1M | 80 mM | 1.5% | 3% | 6.0 |
| E6 | 0.1M | - | 0.2M | 50 mM | 1.4% | 2% | 5.5 |
| E7 | 0.3M | - | 0.5M | 60 mM | 1.0% | 1% | 4.0 |
| E8 | 0.2M | - | 0.1M | 60 mM | 1.0% | 1% | 6.5 |

Figure 1b shows that it was possible to isolate an amplifiable DNA from all 19 samples when using an extraction buffer of the invention.

**Example 2**

**Stool extraction at elevated temperature**

For detection of nucleic acids from particular cells (e.g. bacteria, parasites) or viruses, an extraction of the stool sample at elevated temperatures is expedient in order to ensure efficient release of the DNA.

$10^5$ agrobacteria were added to in each case 1 g of stools and worked up according to the method in Example 1. The stool sample was extracted in a buffer of the invention for 5 min at 4°C, room temperature of 18-25°C (RT), 50°C, 70°C, 80°C or 90°C. The efficiency of lysis was determined via the total DNA yield and the

efficiency of lysis of the added agrobacteria was determined via the amplification of a specific agrobacteria sequence (vir gene). The results are shown below in Table 2.

Table 2

| Temperature | Total DNA yield (ng/µl) | Vir amplification |
|---|---|---|
| 4°C | 115 | + |
| RT | 161 | ++ |
| 50°C | 255 | +++ |
| 70°C | 536 | ++++ |
| 80°C | 521 | ++++ |
| 90°C | 548 | ++++ |

The results are based on in each case two independent stool extractions at the temperature indicated. Total DNA yield was determined via OD measurement at 260 nm. The amplification products were fractionated on an agarose gel. + indicates the efficiency of amplification (+ to ++++: increasing efficiency).

Table 2 shows that both total DNA yield and lysis of bacteria and thus the amplification yield increased markedly when increasing the incubation temperature to at least 50°C, in particular to at least 70°C.

## Claims

1. A method for the purification, stabilization or/and isolation of nucleic acids from biological materials, in which an extraction buffer and an adsorption matrix for binding contaminations are added to the nucleic acid-containing sample and the nucleic acids are subsequently removed from the adsorption matrix,
**characterized in that**
the extraction buffer contains
(a) a pH in the range from 2-8,
(b) a salt concentration of at least 100 mM or/and
(c) a phenol-neutralizing substance.

2. The method as claimed in claim 1,
**characterized in that**
an extraction buffer of pH 4-6.5 is used.

3. The method as claimed in claim 1 or 2,
**characterized in that**
an extraction buffer with KCl or/and NaCl at a concentration of at least 100 mM is used.

4. The method as claimed in any of the preceding claims,
**characterized in that**
an extraction buffer with at least 0.5% polyvinylpyrrolidone as phenol-neutralizing substance is used.

5. The method as claimed in any of the preceding claims,
**characterized in that**
an insoluble carbohydrate-based adsorption matrix is used.

6. The method as claimed in any of the preceding claims,
**characterized in that**
potato flour or components thereof, where appropriate mixed with other carbohydrates, is used.

7. The method as claimed in any of the preceding claims,
**characterized in that**
the nucleic acid-containing sample is taken from feces.

8. The method as claimed in any of the preceding claims,
**characterized in that**
the sample is incubated in the extraction buffer prior to contacting with the adsorption matrix.

9. The method as claimed in claim 8,
**characterized in that**
the incubation temperature is ≤ 10°C.

10. The method as claimed in claim 8,
**characterized in that**
the incubation is carried out under conditions which are beneficial to a release of the nucleic acids.

11. The method as claimed in claim 10,
**characterized in that**
the incubation temperature is ≥ 50°C.

12. The method as claimed in any of the preceding claims,
**characterized in that**
the sample is directed over the adsorption matrix by centrifugation, by applying reduced pressure or/and by means of gravity.

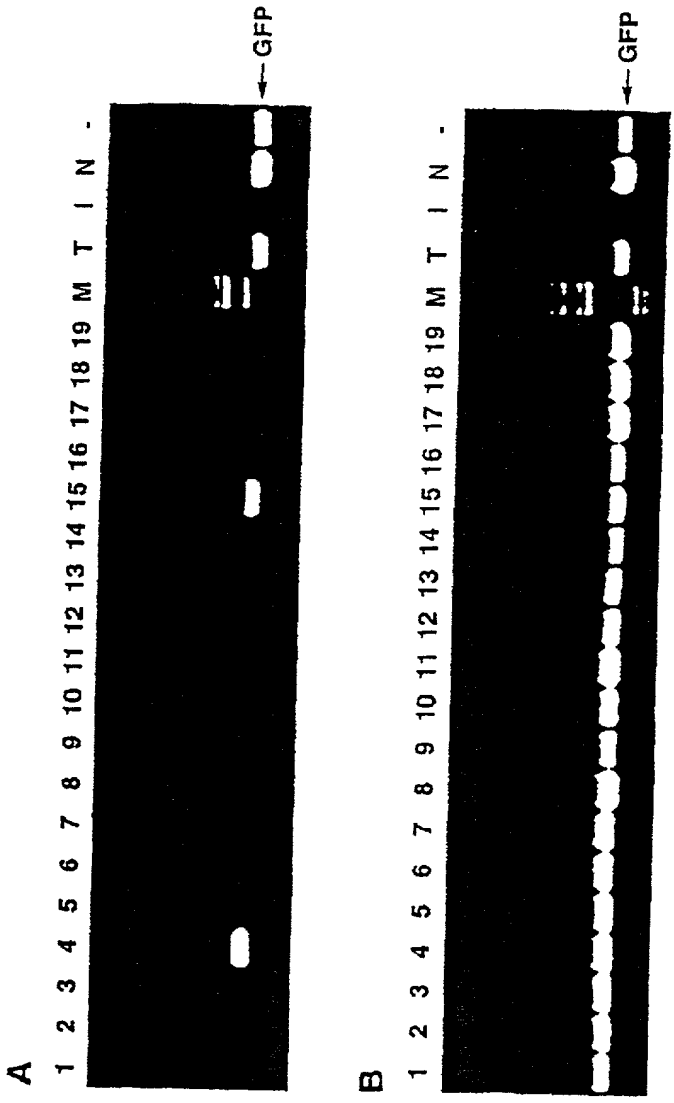
13. The use of a method as claimed in any of claims 1 to 12 for the analysis, detection or isolation or nucleic acids from stool samples.

14. A reagent kit for purification, stabilization or/and isolation of nucleic acids from biological materials comprising:

(a) an extraction buffer as defined in any of claims 1 to 4, which is suitable for taking up a nucleic acid-containing sample, and

(b) an adsorption matrix for binding contaminations of the biological materials.

Figure 1:


A
B
GFP
GFP
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 M T I N -
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 M T I N -

#4

Atty. Docket No. QGN-020.0P US

# COMBINED DECLARATION FOR A PATENT APPLICATION AND POWER OF ATTORNEY

As the below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled

## METHOD FOR ISOLATING DNA FROM BIOLOGICAL MATERIALS

the specification of which:

(check one) [ ] is attached hereto.

[X] was filed as U.S. Ser. No. 09/889,093 on 10-JUL-2001
and was amended on (concurrently with filing) (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I do not know and do not believe that the invention was ever patented or described in any printed publication in any country before our invention thereof or more than one year prior to this application.

I do not know and do not believe that the invention was in public use or on sale in the United States of America more than one year prior to this application.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or (f) or 365(b) of any foreign application(s) for patent or inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's or plant breeder's rights certificate(s), or any PCT international application filed by me on the same subject having a filing date before that of the application on which priority is claimed:

| 199 00 638.5 | DE | 11-JAN-1999 | Priority Claimed [X] Yes [ ] No |
|---|---|---|---|
| (Number) | (Country) | (Day/Month/Year filed) | |

I hereby claim the benefit under Title 35, United States Code, §§ 119 (e) and 120 of any United States Patent application(s) or under § 365(c) of any PCT international applications designating the United States listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or international application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

| | | |
|---|---|---|
| **PCT/EP00/00052** | **05-JAN-2000** | **EXPIRED** |
| (Application Serial No.) | (Filing Date) | (Status: patented/pending/abandoned) |
| | | |
| (Application Serial No.) | (Filing Date) | (Status: patented/pending/abandoned) |

I hereby appoint:

| | |
|---|---|
| Leon R. Yankwich | Registration No. 30,237 |
| Thomas R. Berka | Registration No. 39,606 |
| Kenneth P. Zwicker | Registration No. 43,310 |
| David G. O'Brien | Registration No. 46,125 |

and the firm of Yankwich & Associates, having an office at 130 Bishop Allen Drive, Cambridge, Mass. 02139 as my attorney and attorneys, with full powers of substitution and revocation and full authority to prosecute this application and to transact all business before the U.S. Patent and Trademark Office connected therewith.

**Send all official correspondence to:**

**Leon R. Yankwich, Esq.**
**Yankwich & Associates**
**130 Bishop Allen Drive**
**Cambridge, Mass. 02139**

Direct telecommunications to:

Leon R. Yankwich, Esq.
telephone: (617) 491-4343
telecopier: (617) 491-8801

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Title 18, United States Code, §1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Full name of first inventor **Oliver Müller**

First inventor's signature _____ date

Residence **Dortmund (DE)**
Citizenship **German**
Post Office Address **Harnackstrasse 61a, D-44236 Dortmund (DE)**

#4

Atty. Docket No. QGN-020.0P US

# COMBINED DECLARATION FOR A PATENT APPLICATION AND POWER OF ATTORNEY

As the below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled

## METHOD FOR ISOLATING DNA FROM BIOLOGICAL MATERIALS

the specification of which:

(check one) [ ] is attached hereto.

[X] was filed as U.S. Ser. No. 09/889,093 on 10-JUL-2001
and was amended on (concurrently with filing)
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I do not know and do not believe that the invention was ever patented or described in any printed publication in any country before our invention thereof or more than one year prior to this application.

I do not know and do not believe that the invention was in public use or on sale in the United States of America more than one year prior to this application.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or (f) or 365(b) of any foreign application(s) for patent or inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's or plant breeder's rights certificate(s), or any PCT international application filed by me on the same subject having a filing date before that of the application on which priority is claimed:

| 199 00 638.5 | DE | 11-JAN-1999 | Priority Claimed [X] Yes [ ] No |
|---|---|---|---|
| (Number) | (Country) | (Day/Month/Year filed) | |

I hereby claim the benefit under Title 35, United States Code, §§ 119 (e) and 120 of any United States Patent application(s) or under § 365(c) of any PCT international applications designating the United States listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or international application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

| | | |
|---|---|---|
| **PCT/EP00/00052** | **05-JAN-2000** | **EXPIRED** |
| (Application Serial No.) | (Filing Date) | (Status: patented/pending/abandoned) |
| (Application Serial No.) | (Filing Date) | (Status: patented/pending/abandoned) |

I hereby appoint:

| | |
|---|---|
| Leon R. Yankwich | Registration No. 30,237 |
| Thomas R. Berka | Registration No. 39,606 |
| Kenneth P. Zwicker | Registration No. 43,310 |
| David G. O'Brien | Registration No. 46,125 |

(4)

and the firm of Yankwich & Associates, having an office at 130 Bishop Allen Drive, Cambridge, Mass. 02139 as my attorney and attorneys, with full powers of substitution and revocation and full authority to prosecute this application and to transact all business before the U.S. Patent and Trademark Office connected therewith.

**Send all official correspondence to:**

**Leon R. Yankwich, Esq.**
**Yankwich & Associates**
**130 Bishop Allen Drive**
**Cambridge, Mass. 02139**

Direct telecommunications to:

Leon R. Yankwich, Esq.
telephone: (617) 491-4343
telecopier: (617) 491-8801

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Title 18, United States Code, §1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

1-00

Full name of first inventor **Oliver Müller**

First inventor's signature _____ 1. Oktober 2001
date

DEX

Residence **Dortmund (DE)**
Citizenship **German**
Post Office Address **Harnackstrasse 61, D ~~44236~~ 44139 Dortmund (DE)**

1.Okt.2001

# ASSIGNMENT

We,

(1) Oliver MÜLLER, and

(2) Markus SPRENGER-HAUSSELS

residing, respectively, at

(1) Harnackstrasse 61, D-~~44236~~ 44139 Dortmund (DE), and

(2) Grünstrasse 52, D-42697 Solingen (DE)

both citizens of Germany, for good and valuable consideration, receipt of which is hereby acknowledged, have assigned, sold and transferred to and do hereby assign, sell and transfer to MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG (DER WISSENSCHAFTEN), a corporation organized and existing under the laws of Germany and having an office and a place of business at Hofgartenstrasse 2, D-80539 Munich, Germany, its successors and assigns:

1) the entire right, title and interest in the United States and in all countries throughout the world in and to any and all my/our inventions and discoveries disclosed in U.S. Patent Application Ser. No. 09/889,093, filed **July 10, 2001** and entitled: "METHOD FOR ISOLATING DNA FROM BIOLOGICAL MATERIALS" {Atty. Docket No. QGN-020.0P US (19619P US-WO/WWbj)}, including any renewals, revivals, reissues, reexaminations, extensions, continuations and divisions thereof, and any substitute applications therefor;

2) the full and complete right to file patent applications in the name of Max-Planck-Gesellschaft zur Förderung, its designee, or in our names at the election of Max-Planck-Gesellschaft zur Förderung or its designee, on the aforesaid inventions, discoveries and applications in all countries of the world;

3) the entire right, title and interest in and to any Letters Patent which may issue thereon in the United States or in any other country of the world and any renewals, revivals, reissues, reexaminations and extensions of the same; and

4) the entire right, title and interest in all Convention and Treaty Rights of all kinds thereon, including without limitation all rights of priority in any country of the world, in and to the above inventions, discoveries and applications.

We hereby authorize and request the competent authorities to grant and to issue any and all such Letters Patent in the United States and throughout the world to Max-Planck-Gesellschaft zur Förderung as the assignee of the entire right, title and interest therein, as fully and entirely as the same would have been held and enjoyed by us had this assignment, sale and transfer not been made.

We agree, at any time, upon the request of Max-Planck-Gesellschaft zur Förderung, to execute and to deliver to Max-Planck-Gesellschaft zur Förderung any additional applications for patents for said inventions and discoveries, or any part or parts thereof, and any applications for patents of confirmation, registration and importation based on any Letters Patent issuing on said inventions, discoveries or applications, and divisions, continuations, renewals, revivals, reissues, reexaminations and extensions thereof.

We further agree at any time to execute and to deliver upon request of Max-Planck-Gesellschaft zur Förderung such additional documents, if any, as are necessary or desirable to secure patent protection on said inventions, discoveries and applications throughout all countries of the world, and otherwise to do the necessary to give full effect to and to perfect the rights of Max-Planck-Gesellschaft zur Förderung under this Assignment, including the execution, delivery and procurement of any and all further documents evidencing this assignment, transfer and sale as may be necessary or desirable.

| ASSIGNORS: | DATE OF SIGNING: | WITNESSED BY: |
|---|---|---|
| (1) _____ OLIVER MÜLLER | 1. Oktober 2001 date | (1) _____ Witness: |
| (2) _____ MARKUS SPRENGER-HAUSSELS | 9. Oktober 2001 date | (2) _____ Witness: |

2-00

Full name of second inventor **Markus Sprenger-Haussels**

Second inventor's signature _____ 09.10.01
date

Residence **Solingen (DE)** DEX

Citizenship **German**

Post Office Address **Grünstrasse 52, D-42697 Solingen (DE)**

Full name of third inventor **Helge Bastian**

Third inventor's signature _____
date

Residence **Düsseldorf-Benrath (DE)**

Citizenship **German**

Post Office Address **Benrather Scolßallee 94a, D-40597 Düsseldorf-Benrath (DE)**

Full name of fourth inventor **Stefanie Vollert**

Fourth inventor's signature _____
date

Residence **Hofheim-Lorsbach (DE)**

Citizenship **German**

Post Office Address **Hainerweg 11, D-65719 Hofheim-Lorsbach (DE)**

# ASSIGNMENT

We,

(1) Helge BASTIAN , and

(2) Stefanie VOLLERT

residing, respectively, at

(1) Benrather Schloßallee 94a, 40597 Düsseldorf (DE) , and

(2) Hainerweg 11, D-65719 Hofheim-Lorsbach (DE)

both citizens of Germany, for good and valuable consideration, receipt of which is hereby acknowledged, have assigned, sold and transferred to and do hereby assign, sell and transfer to QIAGEN GmbH , a corporation organized and existing under the laws of Germany and having an office and a place of business at Max-Volmer-Strasse 4, 40724 Hilden, Federal Republic of Germany, its successors and assigns:

1) the entire right, title and interest in the United States and in all countries throughout the world in and to any and all my/our inventions and discoveries disclosed in U.S. Patent Application Ser. No. 09/889,093, filed **July 10, 2001** and entitled: "METHOD FOR ISOLATING DNA FROM BIOLOGICAL MATERIALS" {Atty. Docket No. QGN-020.0P US (19619P US-WO/WWbj)}, including any renewals, revivals, reissues, reexaminations, extensions, continuations and divisions thereof, and any substitute applications therefor;

2) the full and complete right to file patent applications in the name of QIAGEN GmbH, its designee, or in our names at the election of QIAGEN GmbH or its designee, on the aforesaid inventions, discoveries and applications in all countries of the world;

3) the entire right, title and interest in and to any Letters Patent which may issue thereon in the United States or in any other country of the world and any renewals, revivals, reissues, reexaminations and extensions of the same; and

4) the entire right, title and interest in all Convention and Treaty Rights of all kinds thereon, including without limitation all rights of priority in any country of the world, in and to the above inventions, discoveries and applications.

We hereby authorize and request the competent authorities to grant and to issue any and all such Letters Patent in the United States and throughout the world to QIAGEN GmbH as the assignee of the entire right, title and interest therein, as fully and entirely as the same would have been held and enjoyed by us had this assignment, sale and transfer not been made.

We agree, at any time, upon the request of QIAGEN GmbH, to execute and to deliver to QIAGEN GmbH any additional applications for patents for said inventions and discoveries, or any part or parts thereof, and any applications for patents of confirmation, registration and importation based on any Letters Patent issuing on said inventions, discoveries or applications, and divisions, continuations, renewals, revivals, reissues, reexaminations and extensions thereof.

We further agree at any time to execute and to deliver upon request of QIAGEN GmbH such additional documents, if any, as are necessary or desirable to secure patent protection on said inventions, discoveries and applications throughout all countries of the world, and otherwise to do the necessary to give full effect to and to perfect the rights of QIAGEN GmbH under this Assignment, including the execution, delivery and procurement of any and all further documents evidencing this assignment, transfer and sale as may be necessary or desirable.

| ASSIGNORS: | DATE OF SIGNING: | WITNESSED BY: |
|---|---|---|
| (1) Helge Bastian<br>HELGE BASTIAN | 9/10/01<br>date | (1) B. Rogg<br>Witness: |
| (2) R. Vollert<br>STEFANIE VOLLERT | 29109101<br>date | (2) B Rogg<br>Witness: |

Full name of second inventor **Markus Sprenger-Haussels**

Second inventor's signature _____ date

Residence **Solingen (DE)**
Citizenship **German**
Post Office Address **Grünstrasse 52, D-42697 Solingen (DE)**

3-00

Full name of third inventor **Helge Bastian**

Third inventor's signature Helge Bastian 9/10/01 date

Residence **Düsseldorf-Benrath (DE)** DEX
Citizenship **German**
Post Office Address **Benrather Scolßallee 94a, D-40597 Düsseldorf-Benrath (DE)**

4-00

Full name of fourth inventor **Stefanie Vollert**

Fourth inventor's signature R. Vollert 29/09/01 date

Residence **Hofheim-Lorsbach (DE)**
Citizenship **German** DEX
Post Office Address **Hainerweg 11, D-65719 Hofheim-Lorsbach (DE)**

# United States Patent & Trademark Office

Office of Initial Patent Examination -- Scanning Division

Application deficiencies found during scanning:

☐ Page(s)_____ of _____ were not present for scanning. (Document title)

☐ Page(s)_____ of _____ were not present for scanning. (Document title)

☐ ***Scanned copy is best available.*** Declaration pages are misnumbered.

2) Figure 1 is dark